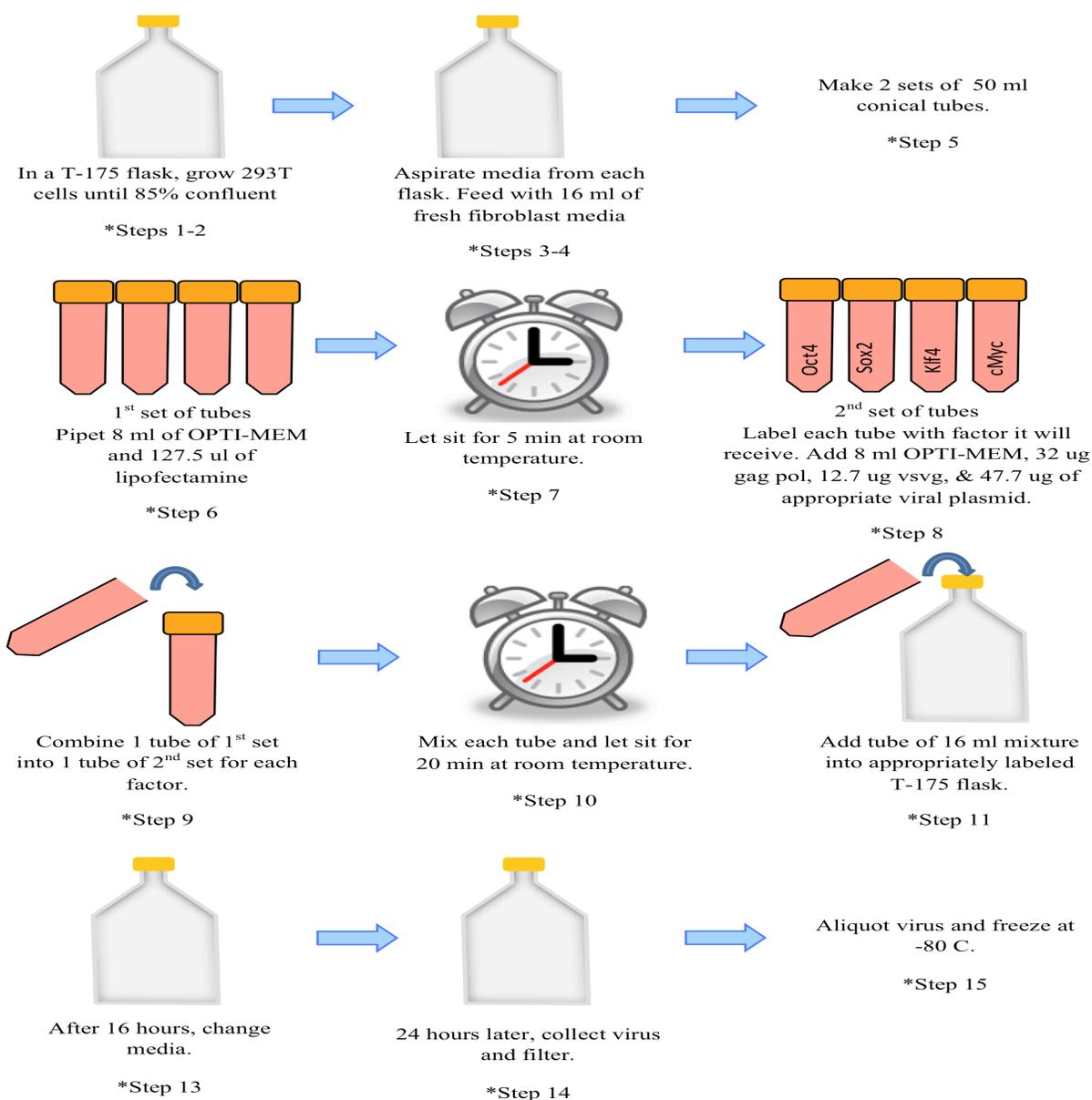


Retrovirus Transfection Protocol	Prepared by: Berggren, Travis tberggren@salk.edu
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Adapted from	Salk Stem Cell Core in-house protocols
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Introduction:

This protocol was developed to consistently produce a high titer retrovirus. This four-factor retrovirus can be used to reprogram fibroblasts into induced pluripotent stem cells.

Flow Chart:



Protocol Steps:

Prepare 293T Cells:

1. Grow 293T cells in a T175 flask. Note: A least 1 T175 flask per factor will be needed, so you must have at least 4 flasks. Each T175 should be fed with 32mls of 293T Media.
2. Cells should be ~85% confluent. Note: Cells are normally ready about 2 days after a 1:5 split.

Transfection of 293T Cells:

3. Aspirate 293T Media from each 293T flask.
4. Feed each flask with 16mls of fresh 293T Media. Tip: Label each flask with which viral factor it will receive (Oct4, Sox2, klf4, or c-myc). *
5. Make 2 sets of 4x50ml conical tubes.
6. For the first set of 4x50ml conical tubes: Give each tube 8mls of OPTI-MEM media and 127.3ul of Lipofectamine 2000.
7. Flick each 50 ml conical tube and let sit for 5 minutes at room temperature.
8. For the second set of 4x50ml conical tubes: Label each tube with a different viral factor (Oct4, Sox2, klf4, or c-myc). Each tube gets 8mls of OPTI-MEM, 32ug of gag pol, 12.7ug of vsvg, and 47.7ug of appropriate viral plasmid.
9. Combine one tube of the first set (OPTI-MEM + Lipofectamine 2000) with 1 tube of the second set (OPTI-MEM + gag pol + vsvg + viral plasmid) for each factor. Each tube will now have 16mls total in it.
10. Mix each tube and let sit for 20 minutes at room temperature.
11. Add the appropriate tube of 16ml mixture to its correspondingly labeled T175 flask.
12. Place in 37C incubator.
13. After 16 hours, change media and replace with 32mls fresh 293T Media. Note: Remember to wear proper personal protective equipment. Some active virus might be present after 16 hours.
14. 24 hours later, collect the 32mls of 293T Media from each T175 flask and filter. This media is now virus rich. **
15. Aliquot virus and freeze at -80C freezer. ***

Materials:

Product	Amount Needed	Supplier	Catalog #
OPTI-MEM	1 bottle	Life Technologies	31985-070
Lipofectamine 2000	1ml vial	Life Technologies	11668-019

50 ml conical tube	8	Corning	430828
T175 flask	4	Corning	431080
293T cells	1 vial	ATCC	CRL-11268
293T media	See below		

293T Medium

Ingredient	Amount (mls)	Supplier	Catalog #
IMDM	85	Life Technologies	12440-053
FBS	15	Gemini or Various Sources ****	100-106
NEAA	1	Life Technologies	11140-050
Glutamax	1	Life Technologies	35050-061

Notes:

* Be very gentle when feeding 293T cells. They will detach from tissue culture treated plastic very easily. We found that culturing 293T cells in a T175 flask allowed us to produce a large amount of virus in a single vessel and to feed 293T cells more gently.

** When using our spinfection protocol there is no need to concentrate the virus.

*** In general, we like to combine the four factors together and aliquot into 4 mls. Each aliquot is then ready to reprogram one well of a 6 well plate when using our spinfection protocol.

****There is lot to lot variation with FBS that contributes to the overall efficiency of virus production using this protocol. It is advisable to screen multiple lots for efficiency of virus production. If lot screening is not an option, then HyClone sourced FBS is typically a safe bet.